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AN AUTORADIOGRAPHIC ANALYSIS OF THE SPECIES SPECIFICITY DURING SPONGE CELL REAGGREGATION [1]

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The early studies of Wilson (1907) indicated that a mixture of sponge cells from two species would separate to form aggregates which were species-specific. Mixed suspensions of cells were seen to form small spherical aggregates which then coalesced with other aggregates of the same species. More recently, the specificity of aggregation has been brought into question by a number of investigators (Curtis, 1962, 1970; Sara, Liaci, and Melone, 1966a, 1966b; Sara, 1968; MacLennan, 1970; Humphreys, 1970a). Sponges have been observed to form bispecific mixtures upon reaggregation after a variety of treatments. This random association of cells may result from a number of causes. For example, dissociation procedures might remove molecules from the surface of the cell which would otherwise confer specificity to it (Humphreys, 1963; Moscona, 1963). Cellular injury might occur due to dissociation or to culturing procedures which could affect aggregation (Curtis, 1962). There is a good possibility that heterospecific cells could be trapped passively in aggregates during the early phases of aggregation. Finally, a cell specific mechanism for aggregation might not exist for many species.

Recently, a method has been developed which shows the species- and tissue-specific nature of freshly dissociated embryonic vertebrate cells. Roth and Weston (1967) developed an "aggregate collection" procedure which has been shown to be a useful tool for analyzing cell specificities in aggregation. The method utilizes monospecific aggregates which have recovered from the stress of dissociation. These aggregates are secondarily confronted with freshly dissociated cells. The results of Roth (1968) have shown that embryonic chick cell aggregates will selectively collect cells of their own genetic or histological type. Because of these results, it is of interest to ascertain whether this same phenomenon can be demonstrated with sponges. The aggregate collection method in this study has been used as a device to show the presence of a specificity which has not been otherwise demonstrated in sponges.

Curtis (1962) pointed out that most studies on specificity had utilized the color of the sponge as the only criterion for species identification in cell aggregates. In many sponges only a small percentage of cells contain pigment; therefore, these studies afforded no means of monitoring behavior in a majority of the cells. The use of radioactive labels is a useful way to overcome this identification problem because the label, when used in small quantities, can allow for a precise identification of all cells without impairing the normal behavior of the cells.

The present study utilizes the aggregate collection method for a series of experi-

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ments in which two unlabeled aggregates are placed into the same suspension of labeled cells. The aggregates compete for the same cells for the same period of time. One aggregate is of the same species as the cells in suspension and the other aggregate is of a different species.

Materials and Methods

Five species of sponge were used for this study: *Haliclona variabilis*, *Haliclona viridis*, *Tedania ignis*, *Homaxinella rudis*, and *Dysidea crawshayi* (DeLaubenfels, 1950). These species were chosen from more than 60 species in Bermuda because they were easily obtained, aggregated well, and were able to survive well under laboratory conditions. They also represent a diversity in taxonomic relationship. Although they are all members of the Class Demospongiae, two are within the same genus; *Haliclona*, *Tedania*, and *Homaxinella* are in separate Orders of the Subclass Monaxonida; and the Genus *Dysidea* is most distantly related to the others since it is in the Subclass Keratosa.

The sponges were collected by hand from Harrington Sound, Bermuda. They were transferred individually to glass jars under water. The jars were sealed and immediately returned to the laboratory. All experiments utilized the fresh material within two hours following collection.

Preparation of the aggregates

Humphreys' procedures (1963) for the dissociation and aggregation processes were used with the following minor modifications. Small pieces (1 cm^3) were cut from a sponge and washed in Millipore filtered seawater. The tissue was pressed through #24 mesh bolting cloth and the dissociated cells were collected in calcium-magnesium–free seawater (CMF-SW). The cells were washed twice in CMF-SW with slow speed centrifugation. They were then resuspended in Millipore filtered seawater containing streptomycin sulfate and sulfadiazine (0.1 mg/ml of each) (MSS-SW). The suspension was diluted to a cell concentration of 5×10^6 cells/ml and 3 ml aliquots of cell suspension were placed into 10-ml culture dishes. These were placed into moisture chambers and the suspensions were rotated at 80 rpm on a shaker at 24° C. Aggregates were harvested after six hours and were selected so that the size of aggregates used in each experiment was initially equal.

Preparation of labeled cells and collection procedures

Cells were disaggregated and washed as described before. They were then resuspended to a final concentration of 20×10^6 cells/ml. in CMF-SW plus ^{3}H-leucine (Schwartz) at a final dilution of 1 μC/ml. ^{3}H-leucine was used instead of other radioactive labels because the cells incorporated it rapidly as opposed to ^{3}H-thymidine which was taken up only slowly by these adult cells. The cells were rotated in plus label for four hours. During this time aggregation was inhibited by the CMF-SW in which the cells and label were suspended. Cell counts after this time indicated that most cells had remained intact and autoradiographs of these cells showed that virtually every cell had incorporated label. After this four hour period, the cells were washed three times in MSS-SW, and then resuspended in MSS-SW to a concentration of 5×10^6 cells/ml. Two aggregates se-

lected for uniform size were placed into each suspension of labeled cells. One aggregate was of the same species as the cell suspension and one was of a different species. These suspensions were rotated at 80 rpm at 24° C in moisture chambers. Replicates were fixed at 6 and 18 hours in Bouin's fixative where they were stored until processing. All combinations of the five species were tested and the results were analyzed by autoradiography.

Histological processing

The aggregates were dehydrated, embedded in paraffin and sectioned at 4 μ. Sections were mounted serially on albumin-coated slides, dried, cleared, and rehydrated to distilled water. They were then soaked for five minutes in cold (0° C) trichloroacetic acid to remove any remaining unbound label. The slides were then rinsed twice in distilled water and processed for autoradiography. The procedures of Kopriwa and Leblond (1962) were followed and were carried out in total darkness. The slides were dipped into Kodak NTB-2 photographic emulsion at 40° C for 3 seconds, drained and dried for 2 hours at 28° C. Coated sildes were stored in dry, light-tight containers at 4° C and were exposed for six weeks. The emulsion was developed with Dektol, stained in 0.1% nuclear fast red and counterstained briefly in 0.2% indigo carmine in saturated aqueous picric acid. Grains were counted under oil immersion using an ocular grid to delineate an overall area of 40 μ^2 at this magnification. Photographs were taken with a Leitz Ortholux camera.

Results

Incorporation of label

The primary objective in labeling the cells was to place a radioactive tag on proteins of each cell. For all five species it was determined that better than 95% of the dissociated cells picked up ^{3}H-leucine under the conditions used. The amount of label picked up by single cells was, however, heterogeneous. In autoradiographs, most cells within a species had from four to fifteen grains over them, but for each species there was a small population of cells which incorporated such a large amount of label that the number of grains was too large to count. The distribution of these cells after collection onto unlabeled aggregates was random although the heavily labeled cells were a constant percentage of the total number of collected cells for a species. No effort was made to determine whether these heavily labeled cells were all of a single histological cell type.

The unlabeled collecting aggregates were closely examined to determine the "background" level in the autoradiographs in areas devoid of labeled cells. Large section areas such as those marked "UA" on Figure 1 were examined for grain distribution. When the number of grains was determined on a per-cell basis, the average for each species was 1.6 grains/cell or less. Table I shows the average number of grains appearing over an entire 40 μ^2 area in the unlabeled aggregates. As can be seen, the number of grains appearing over aggregate sections is related to the kind of cell suspension to which the aggregate was exposed. Thus, more label was introduced into unlabeled aggregates by exposure to *Haliclona variabilis*, *Tedania ignis*, or *Dysidea crawshayi* cell suspensions than by exposure to *Haliclona viridis* or *Homaxinella rudis* suspensions. This introduction of label was essen-

Table I

Background grain counts in unlabeled aggregates derived by exposure to ^{3}H-leucine labeled cell suspensions. Each number represents at least eight counts of 40 μ^2 areas from 6- and 18-hour aggregates

Unlabeled collecting aggregate	Labeled cell suspension				
	Haliclona variabilis	*Haliclona viridis*	*Tedania ignis*	*Homaxinella rudis*	*Dysidea crawshayi*
Haliclona variabilis	53	5	40	13	52
Haliclona viridis	47	17	27	10	49
Tedania ignis	59	15	63	17	75
Homaxinella rudis	63	11	60	18	68
Dysidea crawshayi	50	12	46	16	66
Average	54	12	47	15	62

tially uniform for each kind of cell suspension; that is, *Haliclona variabilis* collecting aggregates did not pick up any more background label from a *Haliclona variabilis* cell suspension than did any of the other four collecting aggregates. Because this incorporation from the cell suspensions was low, at random and essentially uniform throughout, the grains over collection aggregate cells are considered to be "background" for purposes of this study.

It was important to determine whether cells specifically labeled with ^{3}H-leucine would continue to be identifiable for the duration of the experiment. Collecting aggregates from each of the five species were fixed after 6- and 18-hour exposures in each of the five labeled cell suspensions. The autoradiographs of sections from these aggregates were compared by counting the number of grains over cells in labeled and unlabeled areas. In all cases, the small population of cells with exceptionally heavy accumulation of label were excluded from the counts. Table II summarizes the data from this study. First, it can be seen that the number of grains over labeled cells is always much greater than over background cells. By inspection of corrected grain counts in Table II, one can see that there is no significant difference in the number of grains appearing over the comparable cells after 6 and 18 hours. If loss of label due to metabolic turnover were significant, it would be expected that the number of grains over labeled cells would decrease with time, while the background would increase. This was not the case, as is shown in Table II. The difference between background and labeled cells is at least five fold in all cases. This difference is just as apparent after eighteen as after six hours. Because of this large and substantial difference it was possible to identify with great confidence, on a cell to cell basis, the cells in aggregates that were secondarily incorporated from labeled cell suspensions.

Observations on mixtures of cells in suspension

A preliminary study was made in order to observe the aggregation behavior of mixed cell suspensions. On the assumption that mechanisms for species specificity during cell reaggregation might be most pronounced between species with distant

TABLE II

Average grains/cell in labeled and unlabeled areas. Each figure is the average grain number per average cell number per 40 μ² area. Heavily labeled cells are excluded

Unlabeled collecting aggregate	Grains/cell in labeled areas		Background/cell in background areas		Corrected grains/cell in labeled areas	
	6 hr.	18 hr.	6 hr.	18 hr.	6 hr.	18 hr.
Haliclona variabilis	10.7	9.9	1.6	1.3	9.1	8.5
Haliclona viridis	5.8	6.2	0.8	0.9	5.0	5.3
Tedania ignis	14.9	15.0	1.3	1.3	13.6	13.7
Homaxinella rudis	10.7	11.2	0.6	0.7	10.1	10.5
Dysidea crawshayi	15.0	16.0	1.3	1.4	13.7	14.6

taxonomic relationships and less effective between closely related species, these cell suspensions were given special attention. However, no such correlations were observed in the heterospecific combinations used in this study. The same level of species selectivity was observed between closely related species (*Haliclona variabilis—Haliclona viridis*) as was observed between more remotely related species.

When unlabeled cells from any two of the species used were washed with CMF-SW and then mixed in suspension, they were often observed to clump together heterospecifically. After one to two hours, it was no longer possible to determine whether the aggregates were being formed heterospecifically. By this time, each aggregate had assumed a macroscopically recognizable color of one of the two species.

Aggregates formed from mixtures of dissociated cells from two species in which one cell type was radioactively labeled often contained mixtures of labeled and unlabeled cells. For example, when labeled *Haliclona viridis* cells were mixed with unlabeled *Homaxinella rudis* cells and permitted to aggregate for six hours, the green (*Haliclona viridis*) aggregates contained mostly labeled cells with a few unlabeled cells, whereas the red (*Homaxinella rudis*) aggregates were primarily unlabeled, but contained a scattered proportion of labeled *Haliclona* cells. From these preliminary results, it is evident that there is some heterospecific mixing and incorporation of cells even though the aggregates which result are predominately of one species. These results, however, do not show whether this mixing is due to an absence of any species specificity or to a loss of specificity. Also, they do not show whether the clumping which occurs is true aggregation or whether it is a nonspecific response to cellular injury.

Collection of labeled cells by unlabeled aggregates

The pattern of collection of labeled cells by heterotypic aggregates was quite different than that of the homologous collection of cells. Figure 1 is an autoradiograph that shows darkly labeled cells of *Dysidea crawshayi* after collection by a *Dysidea crawshayi* aggregate. As can be seen, the labeled area is integrated within the unlabeled cells of the aggregate. This appearance is characteristic of all five homotypic combinations. At first, the aggregates became almost completely surrounded by labeled cells. Between six and eighteen hours, there is a progressive

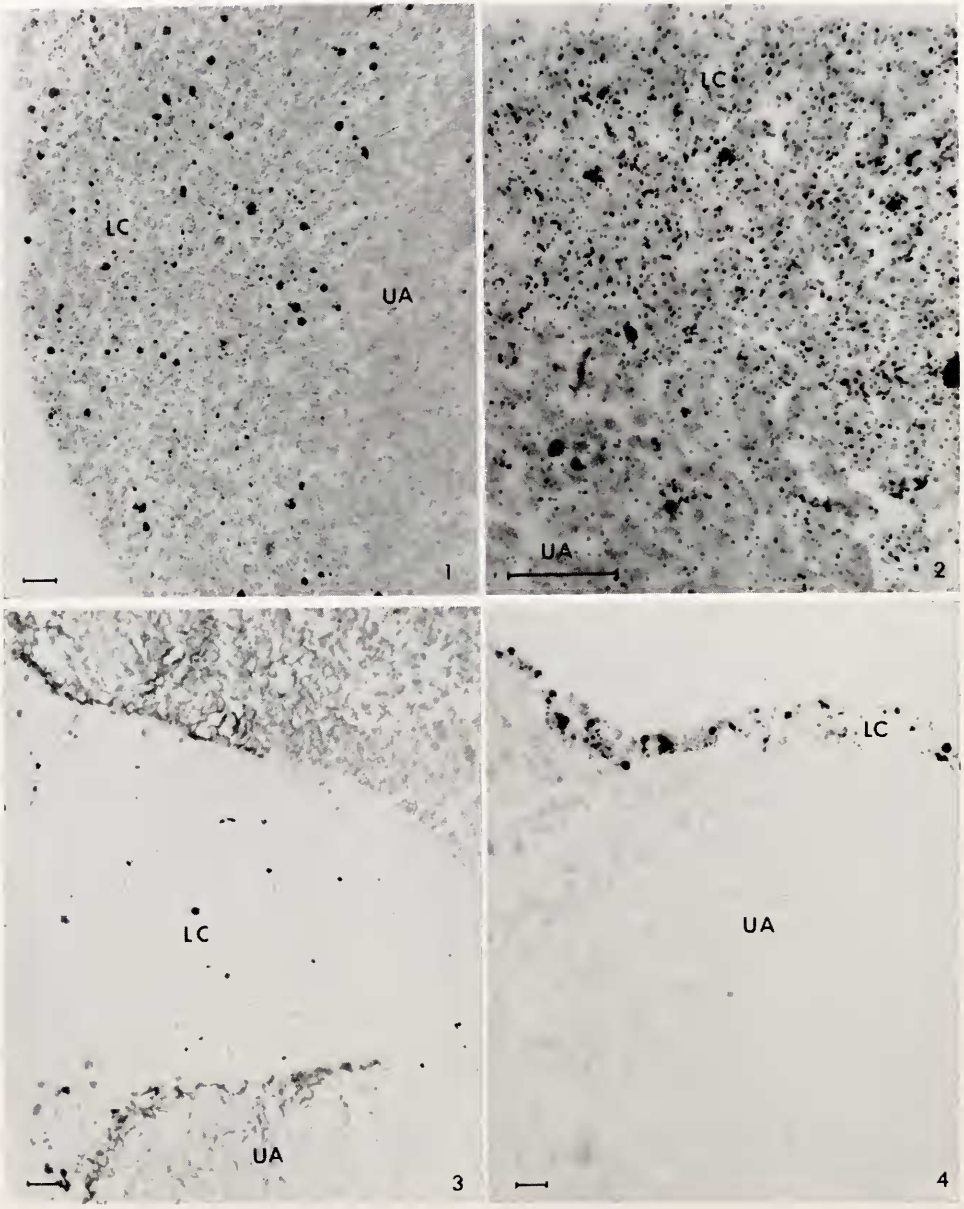

FIGURE 1. Eighteen hour homotypic collection of labeled *Dysidea crawshayi* cells (LC) to an unlabeled *Dysidea crawshayi* collecting aggregate (UA); scale = 10 microns.

FIGURE 2. Eighteen hour homotypic collection of labeled *Homaxinella rudis* cells (LC) to an unlabeled *Homaxinella rudis* aggregate showing the mixing of labeled and unlabeled cells; scale = 10 microns.

FIGURE 3. Eighteen hour heterotypic collection of labeled *Haliclona variabilis* cells (LC) into a folded area of a *Haliclona viridis* collecting aggregate (UA); scale = 10 microns.

FIGURE 4. Eighteen hour heterotypic collection of labeled *Haliclona variabilis* cells (LC) to the surface of an unlabeled *Homaxinella rudis* aggregate (UA); scale = 10 microns.

TABLE III

The distribution of labeled cells on collecting aggregates. (M) mixing of labeled and unlabeled cells. (S) adhesion to the surface of collecting aggregates but no mixing. (O) no labeled cells on aggregates surface. This table summarizes three replicates of aggregate collection experiments

Unlabeled collecting aggregate	Labeled cell suspension				
	Haliclona variabilis	*Haliclona viridis*	*Tedania ignis*	*Homaxinella rudis*	*Dysidea crawshayi*
Haliclona variabilis	MMM	OOS	OOS	SSM	SSS
Haliclona viridis	OOS	MMM	OOO	SSS	OOS
Tedania ignis	SSS	OOS	MMM	SSS	OOS
Homaxinella rudis	OSS	OOS	OOS	MMM	OOS
Dysidea crawshayi	OOS	OSS	OOS	SSS	MMM

mixing of labeled cells with the unlabeled cells of the collecting aggregate. Table III shows the overall results of this autoradiographic examination. The sections were examined for the presence of label and were scored according to three categories: "M," mixing (meaning that the labeled cells were mixing into the unlabeled aggregate), "S," surface adhesion but no mixing, and "O," no collection of labeled cells by aggregates.

Each of the 25 permutations of combinations among aggregates and dissociated cells was repeated three times. In all three replicates, homotypic collections showed labeled cells mixed in among unlabeled cells of the aggregate as is shown by the distribution of autoradiographic label in Figure 2 and in Table III. In only one heterotypic combination was any mixing observed. The exception was found in one of three replicates in which a *Haliclona variabilis* aggregate collected cells of *Homaxinella rudis*. In this case, labeled cells were found in the core of the aggregate. The pattern of aggregate formation for *Haliclona variabilis* and *Haliclona viridis* is such that foreign cells can occasionally be trapped passively in the interior of the aggregate. During the first few hours of aggregation, these species first form small spherical aggregates which fuse to form a flat sheet. This sheet then folds up to form a large sphere. Any cells resting on the surface of such an aggregate can be passively trapped to the inside during the formation of a sphere. Figure 3 shows an example where this has occurred. Cells of *Haliclona variabilis* are partially trapped in the folds of a *Haliclona viridis* aggregate and still there is no mixing. The exceptional case in Table III may have been the result of this kind of entrapment.

Figure 4 shows a typical example of a combination in which heterotypic cells have been collected on the surface of an aggregate. In Figure 4, labeled cells of *Haliclona variabilis* have adhered to the surface of an aggregate of *Homaxinella rudis*. In this, and the other cases such as this, a few labeled heterotypic cells were observed at the periphery of unlabeled aggregates, but the labeled cells were not tightly bound to the unlabeled aggregate. In fact, in many cases where labeled heterotypic cells were present, there was the appearance of a rejection or a separation of the labeled mass from the surface of the unlabeled aggregate as can be seen in Figure 4. With the possible exception of the one case already mentioned, mixing of labeled heterotypic cells with unlabeled collecting aggregates was not observed.

Table IV

Collection of heavily labeled cells per 0.2 mm² area on collecting aggregates at 6 and 18 hours of aggregation. Each number represents the average of nine counts

Unlabeled collecting aggregate	Homotypic collection		Heterotypic collection	
	6 hr	18 hr	6 hr	18 hr
Haliclona variabilis	48	53	15	5
Haliclona viridis	—	—	8	1
Tedania ignis	90	97	31	27
Homaxinella rudis	32	24	2	4
Dysidea crawshayi	40	49	13	9

Most of the cells were collected by the aggregates during the first few hours of aggregation. A study of the sections was carried out to determine whether the number of cells collected increased between six and eighteen hours, or whether collected cells might be lost from the collections. Table IV summarizes the results from counting the number of heavily labeled cells appearing per aggregate section. The heavily labeled cells were easy to recognize and although they constituted only a small proportion of the total number of cells for a species, this proportion was constant for a species. Each number represents the average count for at least nine sections. The sections to be counted were chosen from the largest cross sections of an aggregate and the average count of three adjacent sections was used for each of three replicates. The size of the collecting aggregate varied for each experiment; thus, in order to standardize the counts, the area of the central section of an aggregate was determined. Each figure in Table IV represents the number of heavily labeled cells per 0.2 mm² area in an aggregate section. This number was more difficult to ascertain in heterotypic combinations since the total number of cells collected was small and often these cells were found in surface patches on the collecting aggregates. For the latter cases, counts were made on the three adjacent sections which contained the greatest number of heterotypic cells. *Haliclona viridis* was not included in this study because its percentage of heavily labeled cells was less than 5% of the total number of cells labeled and the values obtained were to low to provide any meaningful data on cell loss. A comparison of 6- and 18-hour radioactive cells in Table IV indicates a trend for cells to be lost from the heterotypic collections and a trend for cells to be added to homotypic collections.

Discussion

This study demonstrates the presence of a species recognition mechanism for dissociated cells of five species of sponge. The data presented here strongly indicate that the specificity of cell recognition, as measured by selective adhesion, may be temporarily weakened by cell dissociation, but within a few hours, species specficity is reestablished and provides an effective isolating mechanism at the level of cell to cell interactions.

Cells were observed to mix nonspecifically during the early stages of aggregation. It would appear that the early stages of aggregation are somehow different

from the processes taking place later in aggregation. Moscona (1965) has described this as the "primary stage" during which random cell associations take place. Sheffield and Moscona (1969) and Sheffield (1970) have studied the primary phase of embryonic chick retina aggregation and have found a random association of histotypic cells during the first one to two hours of aggregation formation. Roth (1968) demonstrated this primary phase indirectly. Using embryonic chick and mouse cells and the aggregate collection system, he found that many more labeled heterotypic cells were picked up by aggregates when unlabeled freshly suspended homotypic cells were included in the suspension, than when heterotypic cells alone were present. There was a random association between the homotypic and heterotypic cells and a specific association between the homotypic cells and the collecting aggregate. This primary phase has been shown in a number of well known studies (Townes and Holtfreter, 1955; Moscona, 1957; Steinberg, 1962), but in these papers, stress was placed on the process of sorting out which demonstrated the return of specificity that was lost or latent during the dissociation procedures.

Nonspecific associations could occur for several possible reasons. Cells may lose specific combining or reactive groups on the cell surface as a result of dissociation; the cells may stick together in response to injury incurred during dissociation; or there might not be a mechanism for specificity during aggregation. Unless cells subsequently sort out, it is difficult to ascertain whether a specific mechanism of recognition exists. Aggregates which do not sort out may normally have a specific recognition system, but experimental conditions might be such that this recognition can not be expressed.

Most reports on the nonspecific aggregation by sponge cells (Curtis, 1962, 1970; Sara *et al.*, 1966a, 1966b; MacLennan, 1970; Humphreys, 1970a) have been based on observations of cells during the early phases of aggregation when an inability to recognize homotypes occurred. This has been observed in the present study. It is possible that these examples represent cases where there is no mechanism for cell recognition. However, one of these reports (Sara *et al.*, 1966b) describes the formation of "mosaics" following bispecific aggregation. It is likely, as shown in the present study, that the mixed aggregates are the result of the primary stage in which no recognition mechanism is present. The cells might re-acquire and demonstrate specificity as in the present results. This acquisition of specificity might be analogous to the sorting out phase which has been observed in vertebrate tissues.

The cases where nonspecificity has been observed in sponges may well be due to the loss of specific surface recognition groups. Studies on enhancement of aggregation (Humphreys, 1963, 1970a; and Moscona, 1963, 1968) have shown that for several species of sponge, a factor can be isolated which enhances aggregation species specifically. These studies indicate that a glycoprotein, lost during dissociation in CMF-SW or Pronase, must be replaced or resynthesized before the cells are able to reaggregate.

Cell injury may play an important part in nonspecific aggregate formation. The cells of *Homaxinella rudis* perhaps best demonstrated this possibility. These cells loosely collected onto heterotypic aggregates in greater proportion than cells of any other species tested. Dissociation of cells with CMF-SW permitted sub-

sequently a greater incidence of mixed agglutinations than did more gentle washing with MSS-SW (the term "agglutination" is used here to distinguish loose cell masses which are easily broken up by pipeting, as opposed to "aggregates" in which adhesions are tighter and tend to resist breakdown by pipeting). As was the case in the early studies of Galtsoff (1929), agglutination often resulted in cytolysis of cells, indicating that cellular injury might have been the factor which caused this agglutination. In their studies on mixed aggregates, both Sara *et al.* (1966b) and Curtis (1962, 1970) used EDTA to dissociate cells. Ball (1966) and Moscona and Moscona (1967) have shown that EDTA has a toxic effect on vertebrate cells. If EDTA were injurious to the sponge cells as Humphreys (1970b) has observed, then the response of the cells might have been a nonspecific "injury" agglutination. If the cells were unable to recover from the treatment, then the nonspecific masses would have remained mixed. Likewise, CMF-SW, trypsin, and other treatments might cause some injury to the cells which could lead to nonspecific agglutinations or aggregations.

The present results show that cells are collected by homotypic aggregates. By six hours, much of this collection is complete. Humphreys (1970b) has pointed out that it might be necessary for only one of two entities (in this case the aggregate) to have a specificity in an aggregate system. The freshly dissociated cell does not have the ability to form a specific association with other cells, but it might respond to an aggregate which has regained specificity. During this time, however, heterotypic aggregation is also taking place. The present results show that the number of cells picked up heterotypically is far less than homotypic collections. This indicates that even if injury were a factor, the adhesions formed by homotypic cells are stronger or more permanent.

The most important adhesions in the present results are those which form first. The vast majority of cells collected to an unlabeled aggregate actually adhere to the labeled cells that were first collected for the simple reason that only one layer can be formed between the aggregate and the collected cells. Subsequent adhesions to collected cells are independent of aggregate influence. Therefore, the specificity demonstrated involves the initial adhesion of cells only. For this reason, the absolute number of cells collected by an aggregate does not reflect the specificity of the cells for the aggregate, but does reflect the stability of the initial adhesions. Adhesive stability may be important in the process which was demonstrated by these experiments. If it can be assumed that layers of cells will continue to add to the collected cells, and given that the shearing force of rotation is present, then the thickness of the collected cell layer reflects the stability of the adhesion between the collecting aggregate and the first layer of cells. If the cell-aggregate adhesion is not as strong as the cell-cell adhesions, or if that former adhesion is gradually lost, then the entire layer of collected cells would tend to peel away from the collecting aggregate as a result of shear forces. The loose patches of heterotypic cells which were observed in these experiments may be indicative of this process. On the other hand, if the cell-aggregate adhesion becomes just as strong as the intra-aggregate adhesions, then it would be expected that cells could be added to the surface continually until shear forces would prevent further addition.

The present results show that the most stable configurations are between homotypes and that the original collecting aggregate surface becomes indistinguishable

as additional homotypic cells are added and move into the aggregate. Therefore, even though nonspecific adhesions may occur early in the aggregate collection process, these adhesions are not as strong nor as stable as those between a cell and an aggregate of the same species. With time and recovery from dissociation, heterospecific cells, if collected, will progressively be lost from a collecting aggregate of a different species.

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Summary

Unlabeled sponge aggregates were placed into suspensions of radioactively labeled sponge cells. All combinations of five species (*Haliclona variabilis, Haliclona viridis, Tedania ignis, Homaxinella rudis*, and *Dysidea crawshayi*) were used for aggregate collection experiments designed to test for species specificity of adhesion. Preliminary experiments had shown that freshly disaggregated cells from any two of the species would co-mingle during early aggregation. The aggregate collection system, however, showed the presence of adhesive specificity for all five species. Labeled and unlabeled cells became mixed when an unlabeled aggregate collected radioactive homotypic cells. Very few labeled cells were collected and mixing was not observed in heterotypic combinations.

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